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Research article

Enterococcus dysbiosis as a mediator of vitamin D deficiency-associated memory impairments

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ABSTRACT

Low vitamin D status is linked to disturbance in cognitive performance. This study explored possible ways how composition and functional capacity of the gut microbiome affects vitamin D metabolism, directing serum vitamin D (VitD) levels and memory impairmets. It was found that gut microbiome composition, characterized by an increase in the relative abundance of Enterococcus and correlated with vitamin D deficiency and, as consequence, with memory impairments. A key mechanism identified in the study was the differential utilization of short-chain fatty acids (SCFAs) produced by gut bacteria as substrates for synthesizing vitamin D3 precursor in the skin. This finding confirms a complex interplay between the gut microbiome, host metabolism, and cognitive health, highlighting the potential significance of targeting Enterococcus dysbiosis in future preventive and therapeutic strategies to address VitD deficiency-related memory impairments. These results underscore the importance of understanding and modulating gut microbiome composition to optimize VitD status and cognitive function.

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1. Introduction

Over the past 10 years, significant information has been gathered on the impact of the microbiome on the central nervous system (CNS), leading to the proposal of the "brain-gut-microbiota axis" concept. The central nervous system (CNS) regulates the permeability, secretion, motility, and immunity of the digestive tract by influencing the enteric nervous system, muscle tissue, and the mucous layer of the intestine through efferent autonomic nervous pathways [1]. Conversely, the intestinal microbiome can impact brain functions via afferent signaling pathways and the secretion of biologically active substances [2,3]. Numerous published studies have demonstrated the effects of intestinal dysbiosis resulting from dietary changes, antibiotic use, non-steroidal anti-inflammatory drugs, or the presence of pathogenic microorganisms—on cognitive brain functions [4,5]. While dysbiosis may not always be a primary trigger, it significantly contributes to the pathogenic process. However, there are contradictory findings in studies investigating alterations in gut microbiota in association with neurodegenerative diseases.

For instance, one study found an increase in the abundance of Ruminococcaceae in Alzheimer's disease (AD) patients compared to healthy controls [6,7], while another study observed a decrease in the relative abundance of *Ruminococcaceae* in AD patients [7,8]. Similarly, some studies have reported decreased levels of *Bacteroides fragilis* in patients with cognitive impairment [9,10], while other studies have found higher or no significant differences in *B. fragilis* abundance between cognitively impaired individuals and healthy controls [6]. Additionally, some studies have observed increased Prevotella species in individuals with cognitive impairment [6], whereas a reduced abundance of Prevotellaceae has been documented in Parkinson's disease (PD) patients [11]. General characteristic signs for several neurodegenerative pathologies are a decrease in the number of *Lachnospiraceae*, with Parkinson's disease and ALS [12], and an increased number of Akkermansia, with PD and Amyotrophic lateral sclerosis [7].

The contradictory findings across various studies may be attributed to the limited statistical power of these investigations and the complex relationship between the gut microbiome and cognitive function. It is also possible that the initial trigger, which launched the pathogenetic process along a particular path, varied among the study participants. However, changes in the intestinal microbiome play a significant role and could serve as an irreplaceable link, the impact of which could halt the progression of the pathological process.

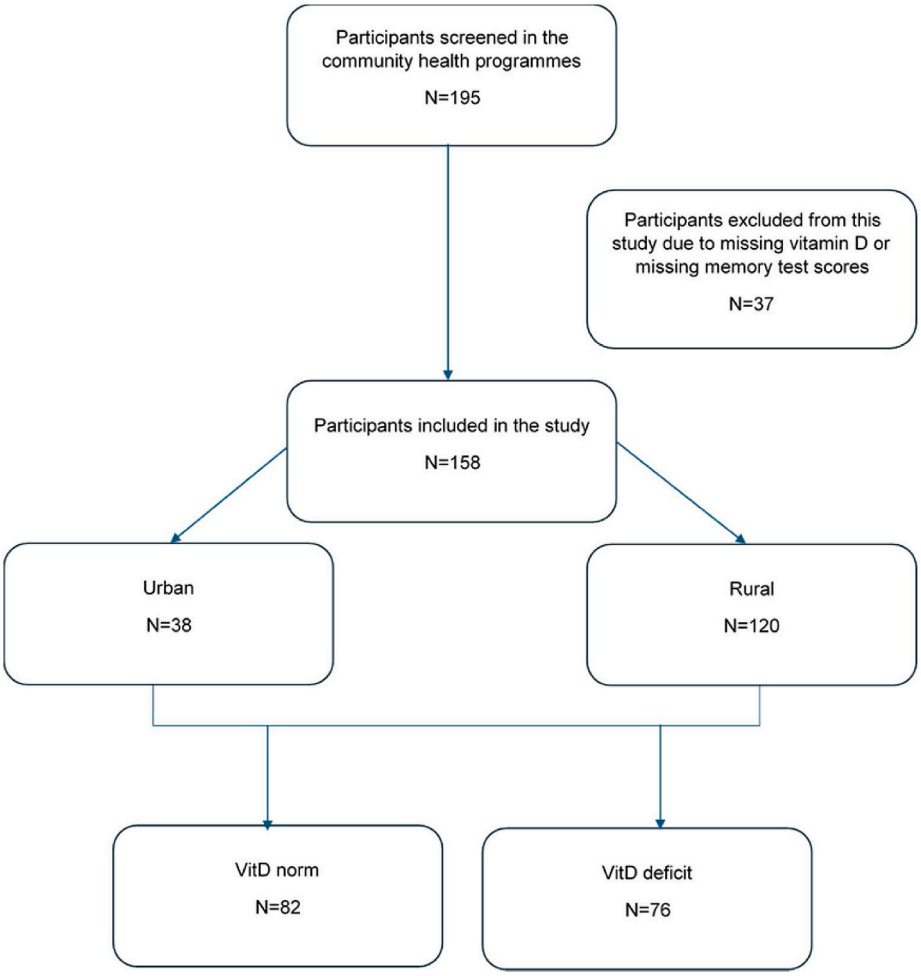
VitD deficiency, a well-documented health concern, has been found to play a significant role in triggering the pathological mechanism of cognitive impairment as well. VitD, a secosteroid hormone, has long been recognized for its pivotal role in regulating calcium homeostasis and bone metabolism [13,14]. However, a wealth of emerging evidence suggests that VitD exerts multifaceted effects on various physiological processes, including cognitive function [15,16,17]. Vitamin D deficiency, a prevalent global health concern, has been increasingly implicated in the pathogenesis of cognitive impairment. Recent published meta-analyses have consistently demonstrated significant correlations between vitamin D deficiency and cognitive decline [18]. May A. Beydoun et al. demonstrated that vitamin D intake was linked to a slower decline in verbal and visual memory [19]. VitD receptors are found in the intestinal epithelium and immune cells, and their activation can influence the composition and metabolic activities of the gut microbiome. Conversely, microbial-derived metabolites may impact VitD synthesis and signaling pathways, highlighting the complex reciprocal interactions between the host and the gut microbiome [20].

Recent studies have made significant strides in unraveling the gut microbiome signatures associated with VitD deficiency and cognitive impairments [21]. These groundbreaking investigations have revealed distinct changes in the abundance of specific bacterial taxa and dysregulation of microbial metabolic pathways in individuals with VitD deficiency and cognitive dysfunction [22,23,24]. While various mechanisms exist through which the microbiome influences the brain's health, we specifically focus on those associated with memory performance and VitD in this work. Our research highlights the complex relationships between serum VitD levels, the composition and functional capacity of the gut metagenome, and memory, considering various environmental factors. By elucidating these interactions, we seek to advance our understanding of the role of VitD and the gut microbiome in cognitive health, potentially paving the way for novel preventive and therapeutic strategies. By elucidating these interactions, we aim to advance our understanding of the role of VitD and the gut microbiome in cognitive health, which could potentially lead to the development of novel preventive and therapeutic strategies.

2. Methods

2.1. Participants

It is a prospective cohort study designed to investigate memory performance in urban and rural populations in Kazakhstan. The study obtained ethical approval from the Local Ethics Committee (LEC) at the National Laboratory Astana (Protocol number 02–2022, dated 01.04.2022, IORG0006963). All participants provided informed consent prior to participation.



Eligible participants were adults aged 18 years and older who were free from acute illnesses, infectious diseases, or cancer pathologies at the time of recruitment. Furthermore, inclusion criteria stipulated that candidates must have abstained from antibiotic, hormone, or probiotic intake for the preceding three months.

2.2. Data collection

Participants completed a paper questionnaire on demographic characteristics, FFQ and ASA-24. Laboratory analyses of VitD were conducted by the Clinical Diagnostic Laboratory Olymp, which has branches in all cities/towns of Kazakhstan. Analyses were performed centrally in compliance with all conditions for the storage and transportation of biomaterial. Stool samples for microbiome studies were collected in DNA/RNA Shield Fecal CollectionTube (Zymo Research, R1101).

2.3. Assessment of memory performance

Memory performance was assessed based on the results of neuropsychological tests[56]. A 10-word list reminder assessed verbal memory and learning. The word lists of 10 common nouns were the same for all participants. Immediate recall was assessed on correctly recalled words summed up over 3 consecutive 1-min trials (range 0–30). C1: Short-term memory assessment (21–30 – normal, 11–20 – moderate memory impairment, <10 – memory impairment). Delayed recall was assessed after the interval during which other tests were introduced (range 0–10). C2: Long-term memory assessment (6–10 – normal, 1–5 - memory impairment).

2.4. Environmental indicators

Indicators of average annual sunshine hours are determined from the map of the Solar Atlas of Kazakhstan. Indicators above sea level are determined according to the map of the Official Topographic Map of Kazakhstan. Humidity indicators, as well as a reconciliation of average annual sunshine hours, are determined in the annual review "Review of the climate features on the territory of

Kazakhstan", prepared by the team of the Scientific Research Center, Hydrometeorological Center, Department of Hydrology, Department of Agrometeorological Monitoring and Forecasting of the RSE "Kazhydromet" [25].

2.5. Metagenomic research

DNA were extracted using ZymoBiomics DNA Microprep (Cat. No: D4300), DNA concentrations were measured using Nanodrop 2000/2000c (ThermoFisher). Sterile water served as a negative control. Following the standard Illumina protocols, samples were sequenced at Novogene (Beijing, China) on the Illumina NovaSeq6000 platform. On average, each metagenomic sample generated 6 Gb of raw data. Sequencing data were processed using the HUMAnN 3.0 workflow. For taxonomic profiling, the MetaPhlAn 4 database version with updated markers and improved taxonomic coverage, mpa_vJan21_CHOCOPhlAnSGB_202103, was used. The average read depth was 29.987 million reads.

2.6. Statistical analysis

Statistical analysis was performed using Python v3.9.16 and R v4.2.2. Taxonomic and functional features with low prevalence (<25 %) were not considered for the analyses. Differential analysis was performed using MaAsLin2 v1.12.0, adjusting for confounding demographic (age, BMI, sex) and location (urbanity, humidity, altitude, and sunshine duration) parameters where appropriate. The Benjamini-Hochberg (FDR-BH) procedure was used to adjust for multiple comparisons. Redundancy analysis (RDA) was performed using vegan v2.6-4. Preliminary regression analysis using GLM was performed using statsmodels v0.13.5. Correlation was calculated using Pearson's r coefficient from Scipy v1.10.1, compositional data was transformed using centered log-ratio (CLR) procedure.

3. Results

3.1. General characteristics of VitD levels in Kazakhstanis

Our study comprised 158 participants, 38 of whom resided in urban areas and 120 in rural areas (Table 1, S1). To determine the average levels among Kazakhstanis and taking into account the diversity of climatic and geographical features [26], we selected 10 recruitment points from Central (latitude 53.206 and longitude 63.457) and Eastern Kazakhstan (latitude 47.578 and longitude 83.674). The primary criterion utilized for the selection of settlements for participant recruitment revolved around the level of ultraviolet radiation index. Across all chosen settlements, this metric consistently ranged between 3 and 5, with uniform fluctuations observed [27,28].

The average vitamin D level among the participants was 21,8 ± 8,9 ng/ml, with insignificant variations across different localities. In our study, a comparison of vitamin D levels between urban (21.3 ± 11.4 ng/ml) and rural (22.0 ± 8.0 ng/ml, $p = 0.41$) populations showed an insignificant difference. However, it is noteworthy that several publications have reported decreased vitamin D levels in rural population [29,30]. There were no statistically significant differences observed in VitD levels in women (22,0 ± 9,1 ng/ml) and men (21,2 ± 8,0 ng/ml) ($p = 0,74$).

At the same time, we observed that certain environmental, lifestyle, and clinical factors are associated with the taxonomic composition of gut microbiota (Fig. 1A) and VitD levels (Fig. 1B–C). Hours of sunshine per year and lower altitude above sea level showed a modest positive association with higher VitD levels (VitD+, $p = 0.09$, $p = 0.01$) (Fig. 1B).

As expected, self-reported VitD intake from the survey (VitD survey) exhibited a positive relationship with circulating VitD concentrations in the group without memory impairment ($p = 0.02$, Fig. 1C). Interestingly, body mass index (BMI) displayed an inverse association with VitD levels in this group ($p = 0.28$), which may be attributed to the influence of adiposity on VitD metabolism and storage.

3.2. Association between VitD levels and memory performance

As measured by various assessments, memory performance (Table 2) showed distinct patterns of association with VitD levels. VitD levels were significantly positively associated with short-term (C1) and long-term (C2) memory performance ($p = 0.005$ and $p = 0.01$) (Fig. 2A–B) in the model adjusted for demographics. This observation highlights the potential importance of VitD in mitigating specific age-related cognitive deficits [31].

Table 1
Baseline characteristics.

Study subgroup	Age, year	p-value	BMI, kg/m2	p-value	VitD, ng/ml	p-value
All (mean ± std)	56,9 ± 10		29,5 ± 6,2		21,8 ± 8,9	
Gender (m/f) 37/121	58,5 ± 6,8/56,4 ± 10,8	0,084	28,0 ± 5,7/29,9 ± 6,3	0,131	21,2 ± 8,0/22,0 ± 9,1	0,748
Urban/rural 38/120	54,7 ± 10,6/57,6 ± 9,8	0,078	28,9 ± 6,7/29,7 ± 6,1	0,327	21.3 ± 11,4/22,0 ± 8,0	0,411
VitD norm/deficit 82/76	55,9 ± 9,3/58,0 ± 10,7	0,185	29,6 ± 5,7/29,3 ± 6,8	0,628	28,5 ± 6,9/14,6 ± 3,5	<0.001

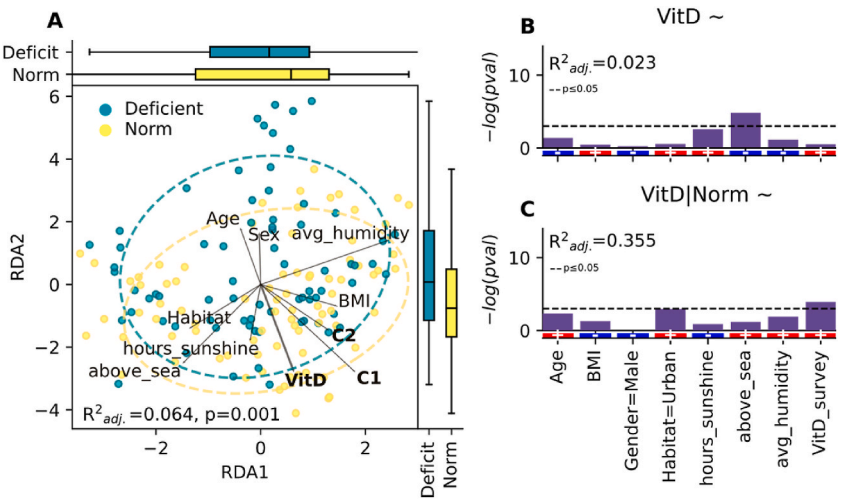


Fig. 1. Environmental and demographic factors. A) Redundancy Analysis (RDA) ordination with biplot; B-C) regression analysis results. B) In all data. C) In the group without memory impairment. Barplots show individual coefficient significance in the model. Dashed line - indicates $p \leq 0.05$ level of significance. C1 = short-term, C2 = long-term memory performance (↑). RDA scaling was set to 2.

Table 2
Memory performance.

Study subgroup	Memory performance, mean of words recalled	p-value
C1 m/f	10,4 ± 7,8/14,5 ± 5,8	0,003
C2 m/f	3,5 ± 2,9/4,9 ± 2,4	0,011
C1 Urban/rural	15,2 ± 5,6/13,0 ± 6,7	0,049
C2 Urban/rural	4,8 ± 2,3/4,5 ± 2,7	0,55
C1 VitD norm/deficit	14,9 ± 5,5/12,1 ± 7,3	0,026
C2 VitD norm/deficit	5,2 ± 2,2/3,9 ± 2,8	0,003

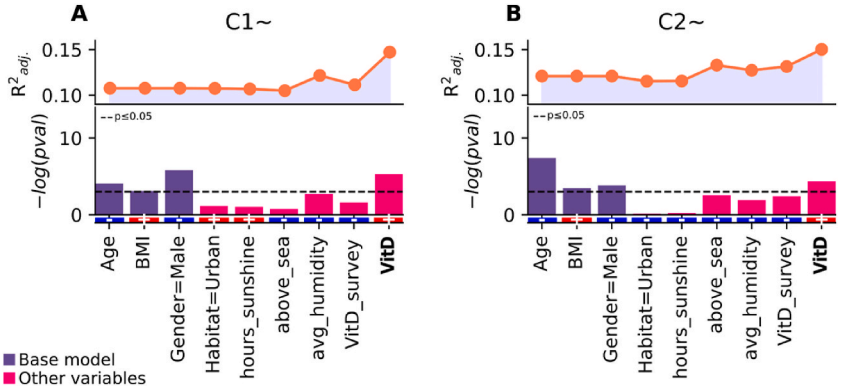


Fig. 2. Results of the regression analysis. Barplot (bottom) shows individual coefficient significance in the baseline model. Lineplot (top) shows overall model performance (adj. R2). Dashed line - indicates $p \leq 0.05$ level of significance. A) short-term memory; B) long-term memory.

3.3. Characterization of gut microbiota compositional profiles

Among all taxonomic levels, only Enterococcus genera showed a significant negative correlation with VitD levels ($p = 0.002$, $q = 0.08$). Interestingly, Enterococcus levels did not show a significant direct association with short-term memory performance (C1 score) or long-term performance. However, it was highly associated with two differentially abundant butyrate producers: Coriobacteriales and Clostridium species (Fig. 3A). Enterococcus was significantly positively associated with Coriobacteriia levels, particularly Senegalimassilia, ($r = 0.21$, $p = 0.008$) and Collinsella, ($r = 0.22$, $p = 0.007$), suggesting a potential cross-feeding relationship between these taxa (Fig. 3B). Coriobacteriia, including Collinsella aerofaciens, are known to produce butyric acid, which serves as a precursor of vitamin D3. Furthermore, Coriobacteriia species were highly associated with the lactose degradation pathway (LACTOSE-DEG,

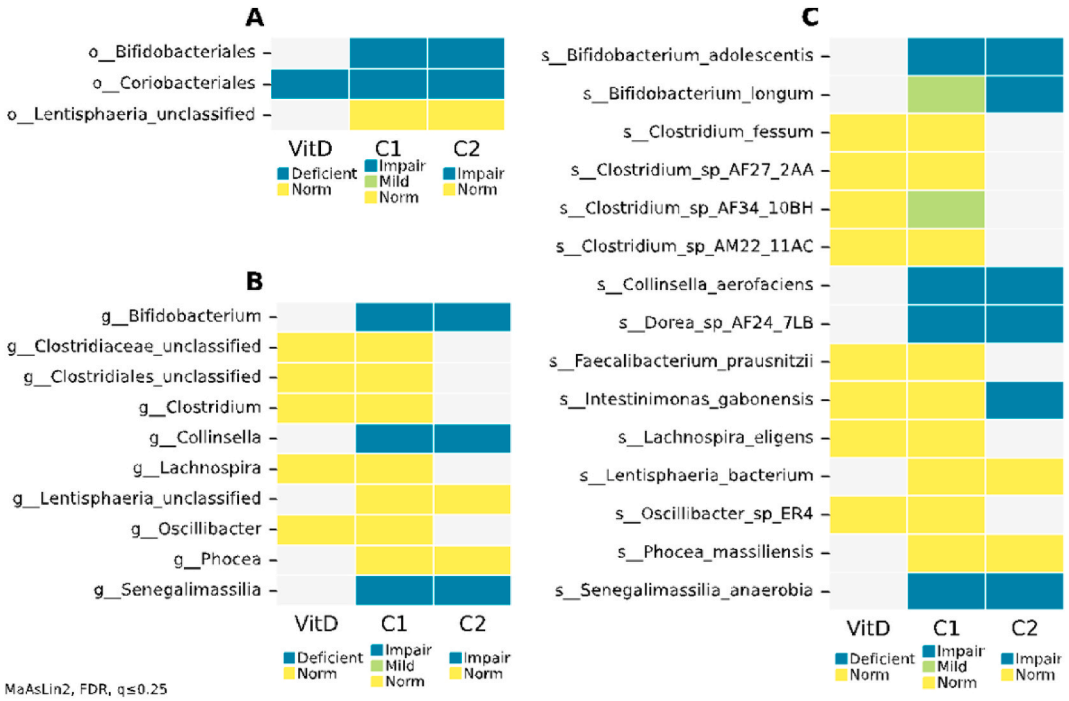


Fig. 3. Association analysis of gut microbiome with VitD levels, short-term (C1) and long-term (C2) memory performance. A) at the order level; B) at the genus level; C) at the species level. Regression analysis (MaAsLin2), FDR, $q \leq 0.25$. Only significant features associated with 2 or more factors are shown. Colors reflect the group with the highest mean abundance.

Senegalimassilia, $r = 0.857$, $p < 0.0001$ and Collinsella, $r = 0.504$, $p = 0.0001$) (Fig. 3C); therefore it cannot be excluded that the observed disturbance in vitamin D metabolism is dependent on impaired lactose fermentation.

At the same time, the broadly accepted butyrate-producing genera Clostridium, which in our study was a marker of VitD and good memory performance, showed a significant negative correlation with *Enterococcus* (Fig. 4A). Specifically, *Clostridium_fessum* exhibited a correlation of −0.17 ($p = 0.03$), and *Clostridium*_sp_AM22_11AC showed a correlation of −0.29 ($p = 0.0002$) with *Enterococcus* levels (Fig. 4B–E). These findings suggest a potential competitive or inhibitory relationship between *Enterococcus* and butyrate-producing *Clostridium* species in the gut microbiome (Fig. 4B). We hypothesized that *Enterococcus* was associated with butyric acid utilization via fatty acid oxidation pathways ($r = 0.36$, $p < 0.0001$), potentially reducing its availability (Fig. 4F).

Furthermore, we have identified gut microbiome representatives correlating with VitD levels and short-term memory performance. These include *Clostridium fessum, Clostridium* sp*_AF27_*2AA*, Clostridium* sp*_AF34_10BH, Clostridium* sp*_AM22_*11AC*, Lachnospira eligens,* and *Oscillibacter* sp*_ER4,* which were significantly increased in groups without VitD deficiency and with normal short-term memory performance (C1 score) (Fig. 4B–E). Notably, several representatives have also been determined to correlate significantly with short-term and long term memory performance but are not directly associated with VitD deficiency. This finding suggests the involvement of other mechanisms unrelated to VitD metabolism in modulating cognitive function.

Of particular significance is *Intestinimonas gabonensis*, which correlates with both vitamin D levels ($coef = 0.654$, $p < 0.02$) and memory performance, including short-term memory ($coef = 1.0$, $p < 0.001$) and long-term memory scores ($coef = 0.878$, $p < 0.003$) (Fig. 3C). *Intestinimonas gabonensis* was first isolated from human faecal samples collected in Gabon, Central Africa, in 2017 [32]; there is currently no evidence of its association with memory performance.

Another significant finding is the correlation of the order of Coriobacteriales with vitamin D deficiency and short-term and long-term memory impairment. In our study, specific representatives at the genus level, such as Senegalimassilia and Collinsella, drive this correlation. However, our analysis did not determine the direct correlation between these genera and VitD deficiency and short-term and long-term memory impairment.

We conducted additional regression analyses using leave-one-out cross-validation without feature selection to identify combinations of parameters that could explain vitamin D (VitD) levels, short-term memory performance (C1), and long-term memory performance (C2) simultaneously using gradient boosting. Our cross-validated regression models demonstrated high accuracy and performance: VitD model ($r = 0.21$, $p = 0.01$), C1 model ($r = 0.43$, $p < 0.0001$), and C2 model ($r = 0.31$, $p < 0.0001$) (Spearman's r). We included demographic and location parameters similar to the MaAsLin2 regression analysis. Notably, our study identified specific taxonomic features that were important across all three regression tasks, based on the overlap of the most critical 100 taxonomic features: *s_Senegalimassilia_anaerobia, s_Bacteroides_caccae, o_Lactobacillales, f_Eubacteriaceae, s_Lachnospiraceae_bacterium, g_Mediterraneibacter, s_Roseburia_intestinalis, f_Oscillospiraceae and s_Ruminococcaceae_unclassified.*

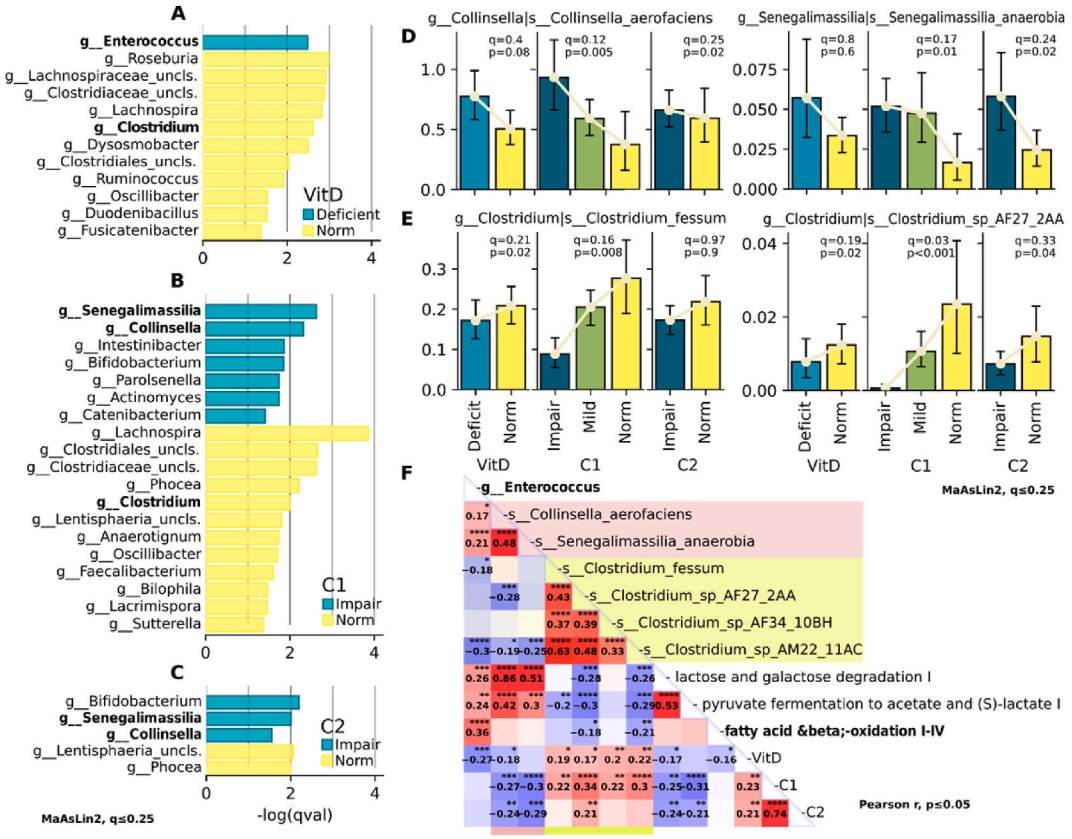


Fig. 4. 2 types of butyrate producers contribute to VitD increase and depletion. Association analysis of gut microbiome with A) VitD levels, B) short-term (C1) and C) long-term (C2) memory performance. Regression analysis (MaAsLin2), FDR, q ≤ 0.25. Significant features associated with 2 or more factors are highlighted. D) Distribution of key differentially abundant taxa - markers of deficiency or norm. E). Fiber-fermenting, butyrate-producing Clostridium species. Regression analysis (MaAsLin2), FDR, q ≤ 0.25. F) Correlation analysis between key differentially abundant parameters. Pearson's r, p ≤ 0.05.

3.4. Functional annotations of gut microbiota

The functional annotations of the gut microbiota have revealed a significant negative correlation between VitD levels and memory, particularly short-term and long-term memory, with the metabolic pathways P124-PWY: Bifidobacterium shunt (c = −0.48, p = 0.009; c = −0.68, p ≤ 0.001; c = −0.68, p ≤ 0.001) and PWY-7254: TCA cycle VII (acetate-producers) (c = −0.53, p = 0.02; c = 0.06, p = 0.81; c = 0.07, p = 0.78) (Fig. 5A). These pathways are involved in energy metabolism and ATP production, and are crucial for memory function [33,34]. The negative associations suggest that alterations in these metabolic pathways, particularly those related to *Bifidobacterium shunt* and acetate production in the TCA cycle, may impact memory performance.

Demonstrated the correlation between VitD levels and memory with patterns of lactate dehydrogenase, pyruvate oxidase and NADP-dependent alcohol dehydrogenase (p ≤ 0.05) (Fig. 5B). The negative correlation between VitD levels and memory is also observed in L-glutamine biosynthesis III (PWY-6549) (c = −0.33, p = 0.001; c = −0.25, p = 0.03; c = −0.16, p = 0.14) and L-lysine biosynthesis I (DAPLYSINESYN-PWY) (c = −0.26, p = 0.04; c = −0.23, p = 0.08; c = −0.25, p = 0.06) (Fig. 5A). These pathways are involved in the biosynthesis of essential amino acids, glutamine, and lysine, respectively [35]. Glutamine, a precursor to the neurotransmitter glutamate, and lysine are pivotal in synthesizing other neurotransmitters, including serotonin, essential for memory processes. Additionally, glutamine and lysine play significant roles in cell signaling pathways and protein synthesis, critical for neurogenesis and synaptic plasticity [36]. A deficiency in Vitamin D, leading to altered amino acid biosynthesis, may indirectly affect these processes, impacting memory function.

We have also found that D-fructuronate degradation pathway (PWY-7242), 4-deoxy-L-threo-hex-4-enopyranuronate degradation pathway (PWY-6507) and D-galacturonate degradation I pathway (GALACTUROCAT-PWY) exhibited positive correlations between vitamin D levels and/or short-term/long-term memory: PWY-7242 (c = 0.11, p = 0.07; c = 0.16, p = 0.01; c = 0.13, p = 0.05); PWY-6507 (c = 0.12, p = 0.08; c = 0.2, p = 0.006; c = 0.16, p = 0.03); (c = 0.12, p = 0.07; GALACTUROCAT-PWY c = 0.19, p = 0.005; c = 0.14, p = 0.03). These metabolic pathways are involved in the degradation of various uronic acid derivatives, particularly fructuronate and galacturonate.

In addition, we observed a high abundance of the O-antigen building pathway, also known as the OANTIGEN-PWY (Fig. 5D), in the

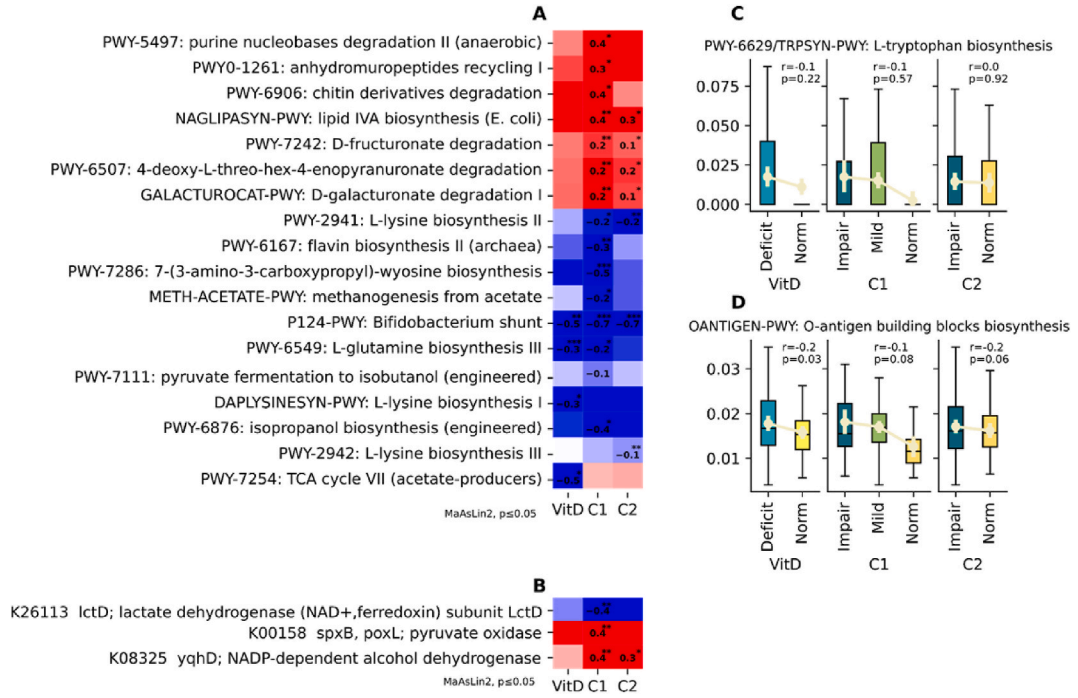


Fig. 5. Association analysis of metabolic pathways with VitD levels, short-term (C1) and long-term (C2) memory performance. A) in short-chain fatty acid-associated pathways from MetaCys database; b) in short-chain fatty acid-associated pathways from KEGG database. Regression analysis (MaAsLin2), $p \leq 0.05$. The heatmap depicts the regression coefficient. C) Relative abundance of L-tryptophan metabolism pathways; D) Relative abundance of O-antigen biosynthesis pathway; Correlation analysis, Pearson's r.

subgroup with VitD deficiency and in subgroups with memory impairments, as evidenced by its presence among the top 30 most discriminative pathways (AUC = 0.6). Our study linked the OANTIGEN-PWY to gram-negative bacteria Succinatimonas sp, Escherichia_coli, Oscillibacter_sp, Megasphaera_elsdenii. Conversely, the positive correlation between Lipid IVA biosynthesis (*E. coli*) (NAGLIPASYN-PWY) and Vitamin D levels/memory (c = 0.24, p = 0.07; c = 0.36, p = 0.009; c = 0.31, p = 0.03) may be attributed to its anti-inflammatory effects and modulation of gut microbiota composition [37,38,39,40].

4. Discussion

In the present study, we have investigated gut microbiome features associated with vitamin D deficiency and memory performance. Our data indicate that the average VitD levels in Kazakhstan are at the lower norm. Vitamin D levels did not significantly differ between urban and rural populations, nor between men and women. Factors associated with higher vitamin D levels include increased hours of sunshine per year and lower altitude above sea level, while higher humidity was linked to lower vitamin D levels. Self-reported vitamin D intake was positively associated with circulating vitamin D concentrations, particularly in individuals without memory impairment. Additionally, higher vitamin D levels were positively correlated with better short-term and long-term memory performance after adjusting for demographic factors. We identified two gut microbiome-mediated ways that may explain vitamin D's effects on memory performance. One way is related to the physiological role of VitD and its receptor VDR in microbiome formation and immune regulation. The second way is associated with synthesizing the vitamin D3 precursor in the skin and the utilization of short-chain fatty acids (SCFAs) produced by intestinal bacteria.

We found that a significant increase in *Enterococcus* species correlates with vitamin D deficiency. In fact, Enterococcus dysbiosis can harm the overall gut microbiome composition and function. This imbalance can lead to the displacement of beneficial bacteria and contribute to various gastrointestinal disorders [41,42,43]. It has been shown that the dominance of Enterococcus species is accompanied by a decrease in the abundance of Roseburia spp. and Clostridium species which are known to be essential producers of butyrate, a short-chain fatty acid (SCFA) with anti-inflammatory properties and a critical source of energy for colonic epithelial cells [44,45]. The decreased butyrate production due to the inhibition of these beneficial bacteria can contribute to impaired gut barrier function, increased inflammation, and the development of various gastrointestinal disorders [46,47].

Furthermore, the imbalance in the gut microbiome caused by *Enterococcus* dysbiosis can lead to other metabolic alterations, such as the accumulation of potentially harmful metabolites, including biogenic amines and secondary bile acid [48]. Enterococcus dysbiosis has been associated with increased oxidative stress in the gut, which can further exacerbate inflammation and damage the intestinal epithelium [49,50]. The extant literature provides compelling support for the potential role of found gut microbiome shifts in the pathogenesis of memory impairment and neurodegenerative disorders.

The consistent findings across multiple studies suggest that the decreased relative abundance of *Roseburia* spp. and non-pathogenic *Clostridium* species in the gut microbiome might contribute to the cognitive deficits observed in Alzheimer's and Parkinson's patients [7,9,51,52]. Similarly, higher abundances of the representatives of *Coriobacteriales*, the genus *Collinsella,* have been observed in individuals diagnosed with neurodegenerative conditions, such as Alzheimer's and Parkinson's [7,51]. In the pathology of amyotrophic lateral sclerosis, there is a significant reduction in the levels of *Oscillibacter* and *Lachnospiraceae* [7].

The described dysbiosis processes suppress the expression of the VDR gene, which is crucial for VitD absorption and regulates the genes encoding proteins responsible for maintaining the integrity of the intestinal barrier and modulating immune responses in the gut [53,54]. This supports dysbiotic changes in the gut microbiome. A decrease in the relative content of fermentation of acetyl-CoA to butanoate II (PWY-5676) associated with the synthesis of butyric acid (butyrate) from acetyl-CoA, is a sign of disruption of the normal microbiota and the development of inflammation. We also observed signs of oxidative stress, such as the accumulation of carbohydrate oxidation products like as fructuronate (PWY-7242: degradation of D-fructuronate).

While the changes in tryptophan metabolism did not demonstrate significance in our study (Fig. 5C), there is evidence suggesting that elevated tryptophan/serotonin levels may contribute to neurodegeneration [55]. Proposed mechanisms include oxidative stress, neuroinflammation, and disruption of normal neurotransmitter balance in the brain [56]. Our study also showed signs of an inflammatory process in a group with memory decline and vitamin D deficiency. One of the main indications of inflammation is the prevalence of OANTIGEN-PWY metabolic pathway, which plays a crucial role in producing the O-antigen part of the lipopolysaccharide (LPS) [57]. In turn, LPS from Gram-negative bacteria can trigger inflammatory responses through Toll-like receptor 4 (TLR4) signalling, potentially contributing to neuroinflammation [58].

Results from functional annotation suggested possible links between vitamin D deficiency and metabolic pathways associated with acetate production in the gut microbiome. Short-chain fatty acids (SCFAs), including acetate, propionate, and butyrate, are produced in the colon through fermentation of dietary fiber by gut microbiota. Our study showed a significant correlation between SCFA and vitamin D levels.

The PWY-7254: TCA cycle VII (acetate-producers) pathway represents the metabolic activities of sppecific gut microbiota capable of producing acetate as a fermentation end-product. This pathway is essential in converting acetate to acetyl-CoA in the colon. Acetate produced by the gut microbiota through the PWY-7254 pathway is then absorbed from the colon and transported to the liver. In the liver, acetate is activated by the acetyl-CoA synthetase, converting it to acetyl-CoA, which serves as the primary precursor for cholesterol biosynthesis [59]. This activation step allows the host cells to utilize the acetate produced by the gut microbiota through the PWY-7254 pathway. The PWY-7254: TCA cycle VII pathway in the gut microbiome is a key contributor to the pool of acetate that further can be used for essential metabolic processes, including synthesizing the precursor molecule for vitamin D, 7-dehydrocholesterol, in the skin [60].

However, several limiting factors need to be addressed, including the sample size and the ability to establish causal relationships. Additionally, the study's location and reliance on a single serum VitD measurement affect the generalizability and robustness of the findings. While the study provides a theoretical explanation for the associations between VitD deficiency, gut microbiome alterations, and metabolic pathways, further investigations, potentially using cell-based or animal models, are essential to validate these proposed mechanisms. Despite these limitations, the study significantly contributes to understanding the complex interplay among VitD, the gut microbiome, and memory performance. Future research efforts should address these limitations to elucidate the underlying mechanisms and implications for human health.

5. Conclusion

This study offers valuable insights into the intricate relationships involving serum vitamin D levels, Enterococcus dysbiosis, gut microbiome composition and function, and memory performance. The findings highlight two crucial gut microbiome-mediated mechanisms that may underly vitamin D's effects on cognitive health: firstly, the physiological role of VitD and its receptor in microbiome formation and immune regulation; secondly, the utilization of short-chain fatty acid (SCFA) substrates produced by gut bacteria for synthesizing vitamin D3 precursor in the skin. The observed Enterococcus dysbiosis associated with VitD deficiency and memory impairments underscores the critical role of specific gut microbiome alterations in disrupting the delicate intestinal ecosystem balance. This microbial imbalance can precipitate the displacement of beneficial bacteria, accumulation of harmful metabolites, increased oxidative stress, and exacerbated inflammation - all of which may synergistically contribute to the development of cognitive deficits and neurodegenerative disorders. These findings provide a comprehensive mechanistic understanding of the gut microbiome-mediated pathways linking VitD and memory performance. The insights gained pave the way for the development of novel preventive and therapeutic strategies aimed at addressing Enterococcus dysbiosis and other gut microbiome imbalances to support cognitive health and reduce the risk of age-related neurodegenerative conditions. Further investigation, including rigorous cell-based and animal model studies, will be crucial to validate the proposed mechanisms and explore their translational potential for improving cognitive outcomes in individuals with vitamin D deficiency.

CRediT authorship contribution statement

Elizaveta Vinogradova: Writing – original draft, Visualization, Software. **Zharkyn Jarmukhanov:** Software. **Madiyar Nurgaziyev:** Methodology. **Alibek Kossumov:** Methodology. **Ayaulym Nurgozhina:** Methodology. **Nurislam Mukhanbetzhanov:** Methodology. **Shynggys Sergazy:** Methodology. **Laura Chulenbayeva:** Methodology. **Argul Issilbayeva:** Methodology. **Sholpan Askarova:** Methodology, Funding acquisition. **Aiym Kaiyrlykyzy:** Methodology. **Saule Rakhimova:** Methodology. **Ulan**

Kozhamkulov: Methodology. **Ulykbek Kairov:** Methodology. **Zhanagul Khassenbekova:** Methodology. **Dinar Tarzhanova:** Methodology. **Ainur Akilzhanova:** Methodology. **Joseph H. Lee:** Writing – review & editing. **Joseph Terwilliger:** Writing – review & editing. **Aliya Sailybayeva:** Methodology. **Makhabbat Bekbossynova:** Resources. **Zhaxybay Zhumadilov:** Resources. **Samat Kozhakhmetov:** Writing – review & editing, Writing – original draft. **Almagul Kushugulova:** Writing – review & editing, Writing – original draft, Supervision, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Data availability statement

The raw shotgun metagenomic sequencing data have been deposited to NCBI under BioProject ID: PRJNA1091163. Upon request, the lead contact can provide any additional information required to reanalyse the data reported in this paper.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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